

## Effect of Emetine on T-2 Toxin-Induced Inhibition of Protein Synthesis in Mammalian Cells

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### ABSTRACT

Chinese hamster ovary cells were used to examine the effect of emetine upon the toxicity of T-2 toxin and several related trichothecene inhibitors of polypeptide synthesis. Emetine inhibited protein synthesis and T-2 toxin-cell association in a concentration-dependent manner. The dose-response curves for these two effects were nearly identical. Over a narrow concentration range (0.3–3.0  $\mu\text{g/ml}$ ), emetine's inhibition of protein synthesis was partially reversible, whereas its inhibition of toxin-cell association was maintained for extended periods. This sustained inhibition of toxin-cell association, resulted in "desensitized" cells with reduced sensitivity to the inhibitory effects of T-2 toxin on

protein synthesis. Similar results were obtained when emetine-preincubated cells were challenged with diacetoxyscirpenol, verrucarin A and roridin A. In contrast, there were no measurable effects of emetine upon the response of the cells to the less potent trichothecenes, deoxynivalenol, T-2 tetraol and verrucarol. In addition to emetine, several other inhibitors of polypeptide synthesis were examined for their effects on T-2 toxin-cell association and sensitivity to T-2 toxin. Of these, only cycloheximide inhibited toxin-cell association. Unlike emetine, sustained protection against the effects of T-2 toxin was not observed with cycloheximide.

T-2 toxin is a potent cytotoxic trichothecene mycotoxin produced by several species of the genus *Fusarium* (Ueno, 1980). T-2 is toxic to both humans and animals and it has been detected at potentially hazardous concentrations in overwintered or improperly stored cereal grains (Hsu *et al.*, 1972; Ohtsubo and Saito, 1977). The toxin has been implicated as a causative agent in moldy corn toxicosis of cattle (Hsu *et al.*, 1972) and in alimentary toxic aleukia (Sato *et al.*, 1975; Ueno, 1980).

Biochemically, T-2 toxin inhibits eukaryotic protein synthesis *in vitro* and *in vivo* (Ueno *et al.*, 1973). Other *in vitro* effects, which have been demonstrated at higher toxin concentrations, include inhibition of mitochondrial respiration (Schiller and Yagen, 1981; Pace, 1983), inactivation of thiol-containing enzymes (Ueno and Matsumoto, 1975) and alterations in cell membrane structure and function (Schappert and Khachatourians, 1983; Gyongyossy-Issa and Khachatourians, 1985). Although the precise relationship between these effects and clinical toxicosis has not been clearly defined, the toxin's most potent and extensively documented effect *in vivo* is its inhibition of protein synthesis.

Studies in animal models have assessed a broad range of drugs for their therapeutic potential in T-2 toxicosis. Although a number of potentially beneficial drugs have been identified from these investigations, most merely prolonged the survival

times of toxin-challenged animals. Exceptions to this were the steroidal anti-inflammatory agents, dexamethasone and prednisolone, which reduced the lethality in mice exposed to a single LD<sub>100</sub> of T-2 toxin (Ryu *et al.*, 1987). Similar protection was noted with superactive charcoal preparations in orally challenged animals, if the charcoal was administered within 3 hr of ingestion of toxin (Galey *et al.*, 1987). Results with ascorbic acid and glutathione prodrugs (Poppenga *et al.*, 1987) have been equivocal and immunological agents such as monoclonal antibodies were of some benefit at lower toxin doses, but were required in relatively large amounts (Feuerstein, 1985).

Although the studies to date have examined a number of strategies for reducing T-2 toxicity, there have been no reports of a pharmacological agent that prevents the cellular accumulation and/or binding of the toxin to its specific receptor once it reaches the target cell. Several unsuccessful attempts were made to protect animals by "occluding" the T-2 receptor using sublethal doses of less potent trichothecenes such as trichodermin (Poppenga *et al.*, 1987) and T-2 tetraol (R. W. Wanne-macher, personal communication). Whereas animal studies provide definitive information on protection, they are sometimes difficult to interpret or control due to the complexities of absorption, metabolism, elimination, etc. of both the toxin and the putative protective drug. In many instances, cultured cells can be an inexpensive and informative system for an initial evaluation of a potentially protective drug. We previously reported detailed studies on the interactions of CHO cells with

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ABBREVIATION: CHO, Chinese hamster ovary.

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radiolabeled T-2 (Middlebrook and Leatherman, 1989a,b,c). Based on that work, we believe that the CHO cell system is an appropriate model for initial evaluation of T-2 protective drugs.

In the accompanying paper, we determined that emetine significantly reduced the cellular accumulation of T-2 toxin (Leatherman and Middlebrook, 1993). Because T-2 toxicity, as measured by inhibition of leucine incorporation, is proportional to the amount of toxin accumulated by the cells (Middlebrook and Leatherman, 1989a), our results suggested that emetine might be protective. This investigation was initiated to determine the efficacy of emetine in diminishing the toxic effect of T-2 (and several related toxins) on protein synthesis in cultured cells. Because emetine is itself a potent inhibitor of polypeptide synthesis (Grollman, 1966; Gupta and Siminovitch, 1976), we have defined conditions for using emetine which permitted a partial recovery from its toxic effect on protein synthesis, while maintaining a long-term depressant effect on T-2 toxin-cell association.

## Methods

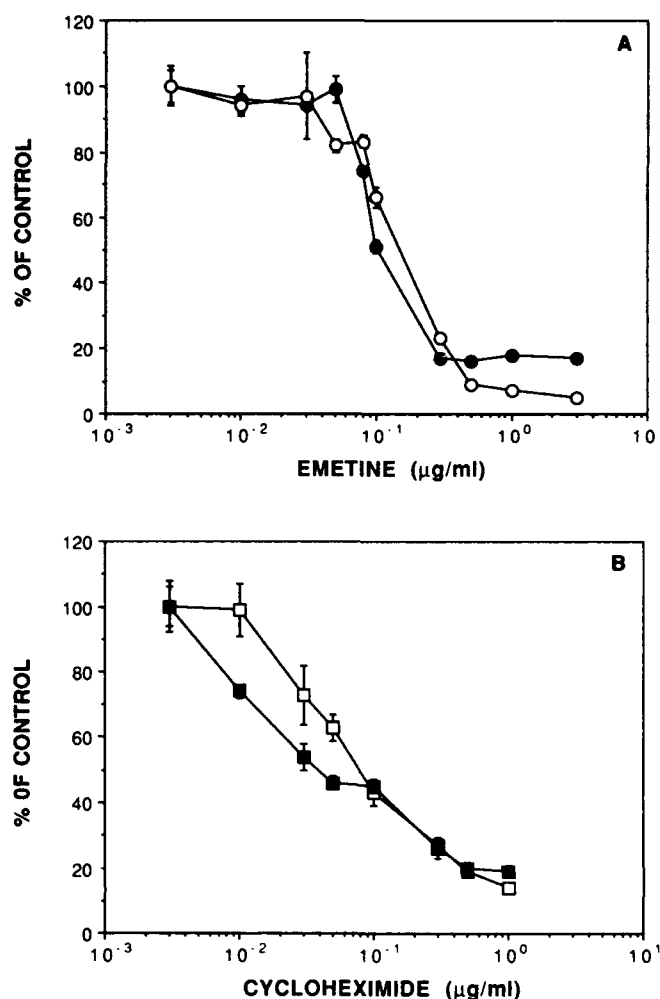
**Toxins, drugs, assays and procedures.** All sources and methods were as described in the accompanying paper (Leatherman and Middlebrook, 1993). Additional trichothecene toxins and cycloheximide were purchased from Sigma Chemical Co. (St. Louis, MO).

## Results

The concentration-dependent effects of emetine on protein synthesis and T-2 toxin-cell association are compared in figure 1A. Cells were incubated with varying concentrations of emetine, then assayed for leucine incorporation or T-2-cell association. Preincubation of CHO cells with increasing concentrations of emetine resulted in reduction of both leucine incorporation and toxin-cell association. Emetine concentrations that inhibited protein synthesis correlated closely with those concentrations that inhibited the cellular accumulation of toxin (fig. 1A). From four independent experiments, the mean  $IC_{50}$  for emetine-induced inhibition of protein synthesis was  $0.10 \pm 0.02 \mu\text{g/ml}$ . This was approximately 2-fold higher than the  $IC_{50}$  for inhibition of toxin-cell association ( $0.05 \pm 0.01 \mu\text{g/ml}$ ; Leatherman and Middlebrook, 1993), but statistical analysis showed no significant difference between the two determinations with  $P = .42$  (nonpaired Student's  $t$  test).

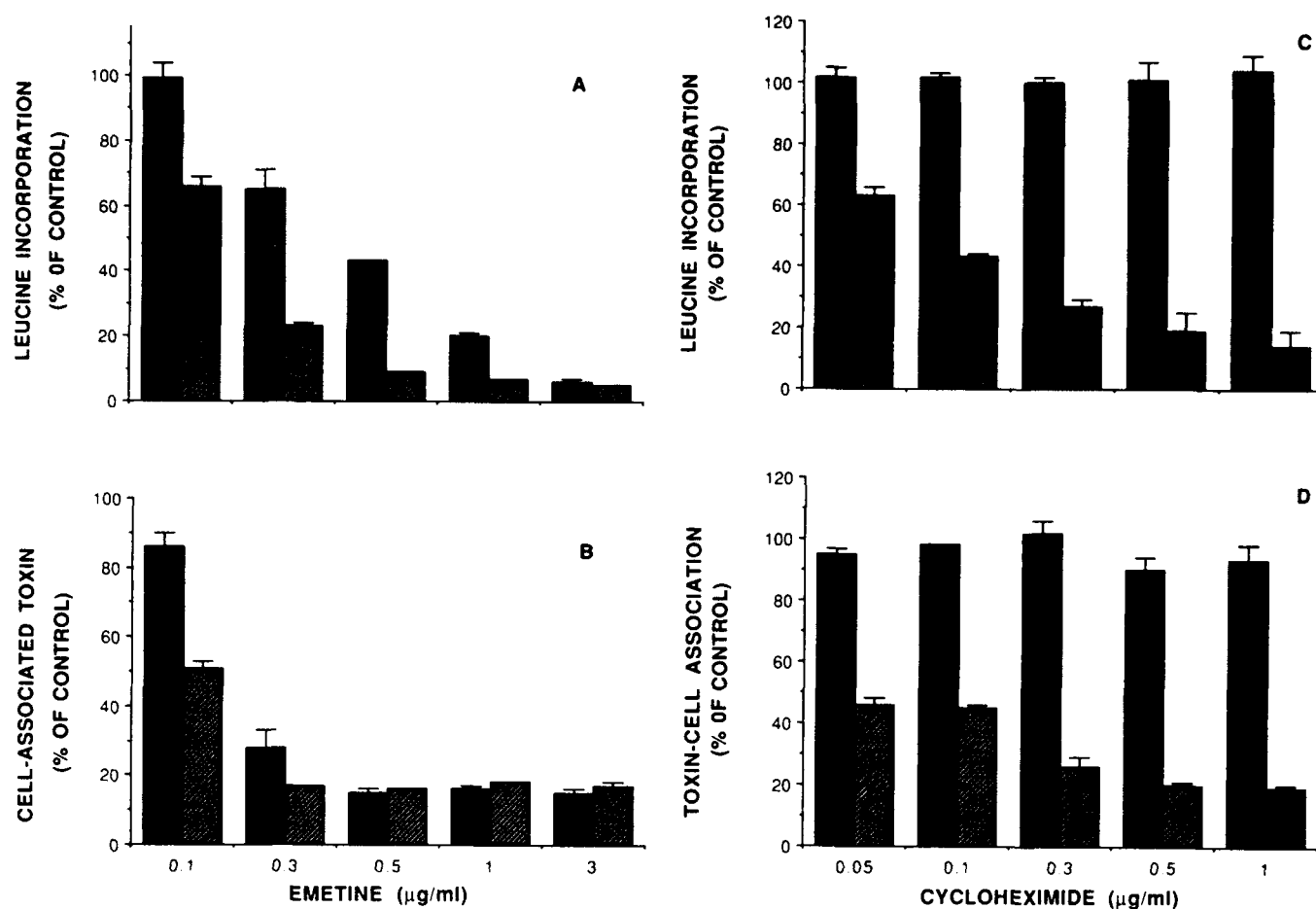
Several other inhibitors of polypeptide synthesis were examined for their effects on toxin cell-association. As shown in figure 1B, preincubation of cells with cycloheximide also produced a concentration-dependent reduction in T-2 toxin-cell association, which correlated with the drug's inhibitory effects upon protein synthesis. However, preincubation with pharmacologically active concentrations of puromycin, sodium fluoride, sodium azide, 2-4-dinitrophenol, 2-deoxy-D-glucose, chloromycetin or oligomycin failed to reduce measurably the accumulation of toxin by CHO cells. To the contrary, the polyribosomal destabilizing agents (i.e., the metabolic inhibitors and puromycin) each had a modest stimulatory effect on toxin-cell association (data not shown).

To examine the reversal of emetine's effects on protein synthesis and toxin-cell association, cells were preincubated with concentrations of emetine which produced maximum reductions in T-2-cell association. Leucine incorporation and toxin-cell association were then measured with or without a 1-hr recovery period in drug-free medium. The data illustrated



**Fig. 1.** Concentration-dependent inhibition of T-2 toxin-cell association and protein synthesis by emetine and cycloheximide. A, emetine: CHO cells were incubated with the indicated concentrations of emetine at  $37^\circ\text{C}$ . After 1 hr, [ $^3\text{H}$ ]leucine or  $0.01 \mu\text{g/ml}$  of [ $^3\text{H}$ ]T-2 toxin was added and the incubation was continued for an additional 1 hr at  $37^\circ\text{C}$ . The cells were then processed for leucine incorporation (○) or for cell-associated toxin (●) as described under "Methods." Values plotted are the means of triplicate determinations with S.E. Control (nonemetine-treated) values were: (○)  $12,300 \pm 650$  dpm; (●),  $26,400 \pm 1,500$  dpm. B, cycloheximide: the cells were incubated with the indicated concentrations of cycloheximide and the experiment was performed as described in A. Leucine incorporation (□), control,  $4,860 \pm 390$  dpm; toxin-cell association (■), control  $23,000 \pm 1270$  dpm.

in figure 2, A and B are from one representative experiment. After a 1-hr incubation with emetine at concentrations between 0.3 and  $3.0 \mu\text{g/ml}$ , toxin-cell association was reduced by approximately 80% and remained maximally depressed after a 1-hr recovery incubation in drug-free medium (fig. 2B). Over the same range of emetine concentrations, leucine incorporation was reduced 80 to 95%, but recovered to various degrees depending upon the emetine preincubation concentration (fig. 2A). With  $0.3 \mu\text{g/ml}$  of emetine, protein synthesis recovered to 68% of control. As the emetine concentration was increased, the recovery of protein synthesis declined to 42% at  $0.5 \mu\text{g/ml}$ , 18% at  $1.0 \mu\text{g/ml}$  and 10% at  $3.0 \mu\text{g/ml}$ . An identical experiment with cycloheximide is shown in figure 2, C and D. In contrast to the emetine results, the depressant effects of cycloheximide on both toxin-cell association and protein synthesis were completely reversible at all concentrations examined.



**Fig. 2.** Recovery of protein synthesis and T-2 toxin-cell association after preincubation with emetine or cycloheximide. A, emetine/protein synthesis: CHO cells were incubated with (solid bars) or without (cross-hatched) the indicated concentrations of emetine at 37°C. After 1 hr, all cells were washed twice with warm Hanks' balanced salt solution (HBSS). Emetine was added to the nonexposed cells and drug-free complete Hanks' medium 199 (H-199) was added to the emetine-preincubated cells. Both sets of cells were reincubated at 37°C. After 1 hr of additional incubation the cells were pulsed with [ $^3$ H]leucine for 60 min, then processed for leucine incorporation as described under "Methods." B, emetine/toxin-cell association: cells were incubated with or without emetine with or without recovery as described in A. After the 2-hr incubation, cells were incubated at 37°C for 1 hr with 0.01  $\mu$ g/ml of [ $^3$ H]T-2 toxin. The samples were then processed for cell-associated toxin as described under "Methods." C, cycloheximide/protein synthesis: the experiment was performed as described in A, but with the indicated concentrations of cycloheximide. D, cycloheximide/toxin-cell association: the experiment was performed as described in B, but with the indicated concentrations of cycloheximide. All values represent the means of triplicate determinations with S.E.

This selective recovery from emetine's effects was examined in six independent experiments and the cumulative data are summarized in table 1. The data show the inhibited and re-

covered values for protein synthesis and toxin-cell association after preincubation with different concentrations of emetine. The mean recovery of protein synthesis was significant at the

**TABLE 1**

**Recovery from emetine effects on toxin-cell association and protein synthesis: statistical summary**

Inhibited and recovered values after incubation with the indicated concentrations emetine are expressed as the percentage of the toxin-cell association or leucine incorporation measured in control (nonemetine-treated) cells. Values are means with S.E. for the data derived from the indicated number of experiments. The statistical differences between inhibited and recovered values were analyzed using a paired Student's *t* test and are listed adjacent to the recovered values for both toxin-cell association and leucine incorporation. At each emetine concentration, recovery ratios were developed by dividing the recovered values by the inhibited values. The recovery ratios were compared for recovery of leucine incorporation and toxin-cell association using the paired Student's *t* test.

Emetine $\mu$ g/ml	Number of Experiments	Protein Synthesis				Toxin-Cell Association			
		Inhibited	Recovered	P	Ratio	Inhibited	Recovered	P	Ratio
0.1	4	70.0 $\pm$ 11.2	96.0 $\pm$ 5.4	.03	NA	42.0 $\pm$ 7.0	79.5 $\pm$ 3.5	.002	2.08 $\pm$ .38
0.3	6	18.3 $\pm$ 1.7	69.3 $\pm$ 4.7	.0001	3.96 $\pm$ .35*	19.3 $\pm$ 2.8	26.5 $\pm$ 4.5	.04	1.35 $\pm$ .12
0.5	6	10.5 $\pm$ .92	43.8 $\pm$ 3.9	.0002	4.28 $\pm$ .39*	19.5 $\pm$ 2.5	18.5 $\pm$ 2.5	.52	.93 $\pm$ .06
1.0	6	7.0 $\pm$ .63	28.0 $\pm$ 3.7	.0002	4.40 $\pm$ .58*	19.5 $\pm$ 1.7	15.0 $\pm$ 2.2	.02	.78 $\pm$ .07
3.0	4	4.8 $\pm$ .25	10.3 $\pm$ 1.7	.05	2.17 $\pm$ .33**	23.5 $\pm$ 3.2	19.0 $\pm$ 3.0	.02	.82 $\pm$ .05

\*  $P < .01$ .

\*\*  $P < .05$ .

$P < .001$  probability level after incubation of cells with emetine at concentrations between 0.3 and 1.0  $\mu\text{g/ml}$ . In contrast, recovery of toxin-cell association after pretreatment with 0.3  $\mu\text{g/ml}$  of emetine was marginal and only significant at the  $P = .05$  level. With 0.5 and 1.0  $\mu\text{g/ml}$  of emetine, there was no recovery of toxin-cell association. Recovery ratios were also calculated and compared for recovery of synthesis and toxin-cell association. At emetine concentrations between 0.3 and 1.0  $\mu\text{g/ml}$ , the recovery of protein synthesis was significantly different from the recovery of toxin-cell association with  $P$  values  $< .01$ .

To determine the duration of the depressant effect on toxin-cell association after the removal of emetine, cells were exposed to emetine and allowed to recover under drug-free conditions for variable times at  $37^\circ\text{C}$  (table 2). Toxin-cell association was then measured at pharmacologically active concentrations of T-2. These data showed that, with no recovery period, emetine reduced toxin-cell association to approximately 20% of that measured in control untreated cells. This level of inhibition was maintained for as long as 8 hr after emetine removal.

To determine whether emetine-induced reduction of T-2-cell association was accompanied by a corresponding reduction in T-2 toxicity, cells were preincubated with emetine, washed, allowed to recover and challenged with T-2 toxin. In cells which had not been preincubated with emetine, the rate of leucine incorporation declined with increasing T-2 toxin concentrations (fig. 3A). After a 3-hr total incubation with toxin, leucine incorporation was 79% of control at 0.005  $\mu\text{g/ml}$  of toxin, 35% at 0.008  $\mu\text{g/ml}$ , 24% at 0.01  $\mu\text{g/ml}$  and 2% at 0.03  $\mu\text{g/ml}$ . When emetine-pretreated cells were challenged with T-2 (fig. 3B), concentrations of toxin from 0.003 to 0.01  $\mu\text{g/ml}$  had no measurable effect on the rate of leucine incorporation relative to controls. Leucine incorporation was 95 to 105% of controls at all toxin concentrations except 0.03  $\mu\text{g/ml}$  where incorporation was 38%. Thus, this treatment with emetine produced cells with reduced sensitivity to the inhibitory effect of T-2 on protein synthesis and are hereafter referred to as "desensitized."

The  $\text{ED}_{50}$  for T-2 inhibition of protein synthesis in desensitized CHO cells was determined from dose response curves. The data from one representative experiment are shown in figure 4A. The dose-response curve for desensitized cells was shifted to the right relative to the control. The mean  $\text{ED}_{50}$  values obtained from four separate experiments were  $9 \pm 1$  ng/ml and  $36 \pm 3$  ng/ml in control and desensitized cells, respectively ( $P = .003$ ). Similar experiments with cycloheximide showed no effects on the response of the cells to T-2 toxin (fig. 4B). Note that the data illustrating the effects of emetine were normalized. This was done because the recovery of protein synthesis was never 100% after preincubation with effective

TABLE 2

Duration of emetine's depressant effects on T-2 toxin-cell association

At each toxin concentration, values are the means of triplicate determinations with S.E. expressed as the percentage of toxin-cell association measured in control (nonemetine-treated) cells for each recovery period.

Toxin Concentration ng/ml	Recovery Time hr			
	0	1	4	8
3.0	17 $\pm$ 1	14 $\pm$ 1	15 $\pm$ 1	20 $\pm$ 2
5.0	16 $\pm$ 1	17 $\pm$ 4	16 $\pm$ 1	17 $\pm$ 1
10.0	20 $\pm$ 1	17 $\pm$ 1	18 $\pm$ 1	17 $\pm$ 1
20.0	19 $\pm$ 1	17 $\pm$ 2	22 $\pm$ 0	24 $\pm$ 1

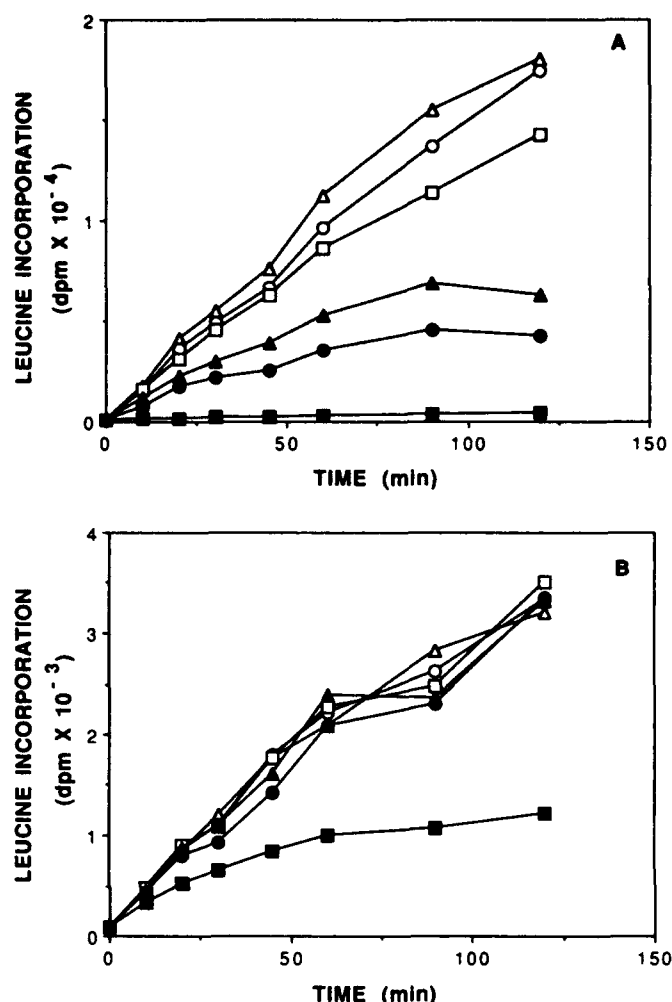
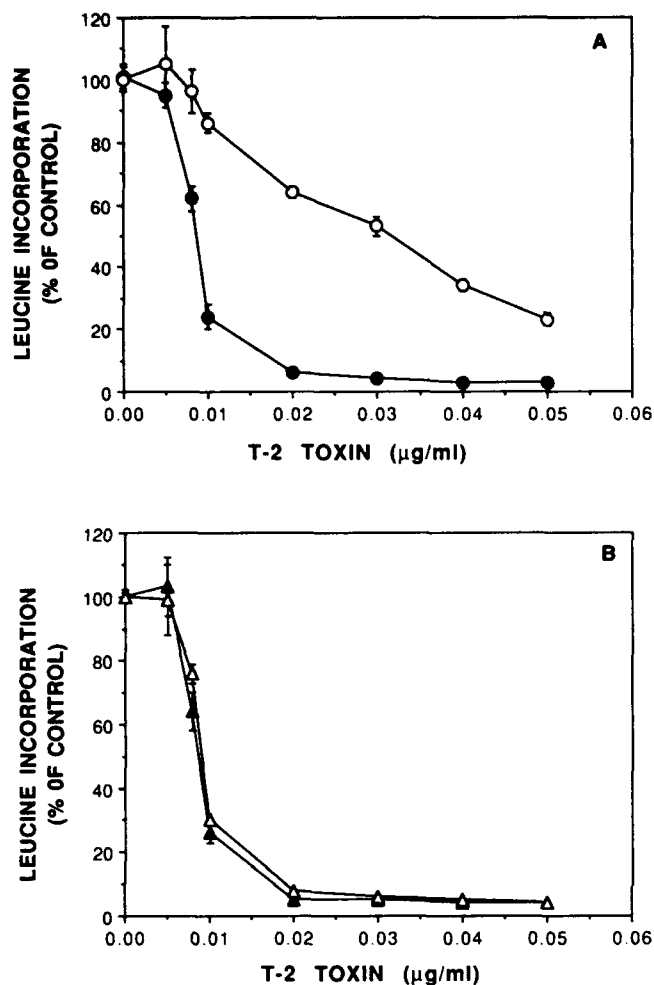


Fig. 3. Effect of T-2 toxin on the kinetics of leucine incorporation in emetine-desensitized and non-desensitized cells. Cells were incubated without (frame A) or with (frame B) 1.0  $\mu\text{g/ml}$  of emetine at  $37^\circ\text{C}$ . After 1 hr, the cells were washed twice with warm HBSS and reincubated in complete H-199 for an additional hour at  $37^\circ\text{C}$ . T-2 toxin was then added to the cells at the following concentrations: 0.0  $\mu\text{g/ml}$  ( $\Delta$ ), 0.003  $\mu\text{g/ml}$  ( $\circ$ ), 0.005  $\mu\text{g/ml}$  ( $\square$ ), 0.008  $\mu\text{g/ml}$  ( $\blacktriangle$ ), 0.01  $\mu\text{g/ml}$  ( $\bullet$ ) or 0.03  $\mu\text{g/ml}$  ( $\blacksquare$ ). After 1-hr incubation with toxin, [ $^3\text{H}$ ]leucine was added to the cells and incubation was continued at  $37^\circ\text{C}$ . At the indicated times, the samples were processed for leucine incorporation as described under "Methods." Data points are the averages of duplicate determinations. Recovery of protein synthesis was 20%.

concentrations of emetine. As a result, toxin-inhibited values for leucine incorporation were expressed as a percentage of the leucine incorporation in the appropriate no-toxin control.

The effect of emetine on the toxicity of six additional trichothecene toxins was examined and the data are presented in figure 5. Emetine desensitization resulted in a 3- to 4-fold shift in the  $\text{ED}_{50}$  for inhibition of protein synthesis by diacetoxyscirpenol, verrucarrin A and roridin A (fig. 5, A-C). In contrast, there was no measurable shift in the response of cells to T-2 tetraol, deoxynivalenol and verrucarol (fig. 5, D-F).

As noted earlier, the data in figures 4 and 5 were normalized to permit easy visualization of changes in  $\text{ED}_{50}$ . Examination of the primary data reveals that normalization overstates the effect. At low toxin concentrations, absolute levels of leucine incorporation were higher in emetine-untreated cells, because recovery from emetine's effect on protein synthesis was never



**Fig. 4.** Concentration-dependent inhibition of protein synthesis by T-2 toxin in cells preincubated with or without emetine and cycloheximide. **A**, emetine: cells were incubated with (○) or without (●) 0.5 µg/ml of emetine for 1 hr at 37°C. After 1 hr, the cells were washed twice with warm HBSS and reincubated in complete H-199 at 37°C for an additional 1 hr. The indicated concentrations of T-2 toxin were then added to both control and desensitized cells for 30 min followed by a 30-min pulse with [<sup>3</sup>H]leucine. Recovery of protein synthesis after emetine preincubation was 42%. **B**, cycloheximide: the experiment was performed as described in **A**, except the cells were incubated with (□) or without (■) 1.0 µg/ml of cycloheximide for 1 hr in lieu of emetine. All data points are the means of duplicate determinations.

complete. However, as toxin concentrations approached maximally toxic doses, the desensitizing effects of emetine became protective, as illustrated in figure 6. For T-2, verrucarins A, diacetoxyscirpenol and roridin A, leucine incorporation in desensitized cells was 3- to 5-fold higher than that in control cells challenged with the same concentrations of toxins. In contrast, leucine incorporation was nearly identical in control and desensitized cells incubated with maximally effective concentrations of deoxynivalenol or T-2 tetraol.

### Discussion

Specific therapies for T-2 toxicosis are few in number and limited in efficacy. Depending upon the conditions of exposure, current therapeutic strategies include general supportive measures in conjunction with a combination of pharmacological agents for reducing absorption of the toxin, alleviating the

shock syndrome, accelerating the metabolism of the toxin and/or increasing the rate of excretion. This therapeutic regimen could be substantially improved by the addition of an agent which acts specifically at the molecular level to inhibit the interaction of T-2 with its target cell receptors.

Emetine is an alkaloid drug with an extensive clinical history. Although emetine can be toxic, it has been used as a therapeutic agent in a number of clinical applications, such as the treatment of bacterial and viral infections of the urinary tract, ocular viral and inflammatory diseases, alcoholism, tumors, multiple sclerosis and scorpion stings (Brossi *et al.*, 1971). A number of mechanisms have been proposed to explain emetine's therapeutic activities, including effects on folic acid utilization (Brossi *et al.*, 1971), the cytoskeletal system (Antoni *et al.*, 1986), protein and nucleic acid synthesis (Grollman, 1968) and a variety of structural, electrophysiological and metabolic effects which are discussed thoroughly by Yang and Dubick (1980). At present, emetine is used primarily as an emetic and as an amebicidal agent in the treatment of parasitic infections of the liver and gastrointestinal tract (Entner and Grollman, 1973; Yang and Dubick, 1980).

In the present investigation, we examined emetine for its effect upon the expression of T-2 toxicity in cultured mammalian cells using the toxin's inhibition of protein synthesis as a measure of T-2 toxicity. Emetine reduces the cellular accumulation of toxin by apparently interfering with the binding of the toxin to its ribosome receptor (Middlebrook and Leatherman, 1993). Although there are extensive clinical precedents for the use of emetine, the therapeutic potential for this drug has always been limited by its acute toxicity which is, to some extent, secondary to emetine's inhibition of macromolecular synthesis (Yang and Dubick, 1980; Dubick and Yang, 1981). The successful utilization of this drug as a therapeutic agent requires a knowledge of the mechanism of its toxic effects and their relationship to the desired therapeutic effect. By carefully defining the determinants for the reversal of emetine's effects, we separated the toxic effects of emetine on protein synthesis from its depressant effects on T-2 toxin-cell association in a cell culture model.

The data presented in figure 1 demonstrate that emetine was essentially equipotent in its inhibitory effects on both toxin-cell association and protein synthesis in CHO cells. Inasmuch as the effects of emetine on both protein synthesis and T-2-cell association appear to be mediated at the ribosomal level (Grollman, 1968; Leatherman and Middlebrook, 1993), it is not surprising to observe a close relationship between the two responses. However, eight other inhibitors of protein synthesis (representing a structurally and functionally diverse collection) were examined for their effects on toxin-cell association, and only one, cycloheximide, produced similar depressant effects on the cellular accumulation of toxin. These data indicate that although there may be a close relationship between emetine's effects on synthesis and toxin-cell association, inhibition of toxin-cell association was not a generalized nonspecific effect associated with any inhibitor of protein synthesis.

Because the maximum reduction in toxin-cell association by emetine was accompanied by near maximal reductions in leucine incorporation, the strategy for using emetine as a desensitizing agent focused on the reversibility of the two effects. There is a considerable amount of disagreement in the literature concerning the reversal of emetine's effects on protein synthesis. In early investigations, Grollman (1968) reported

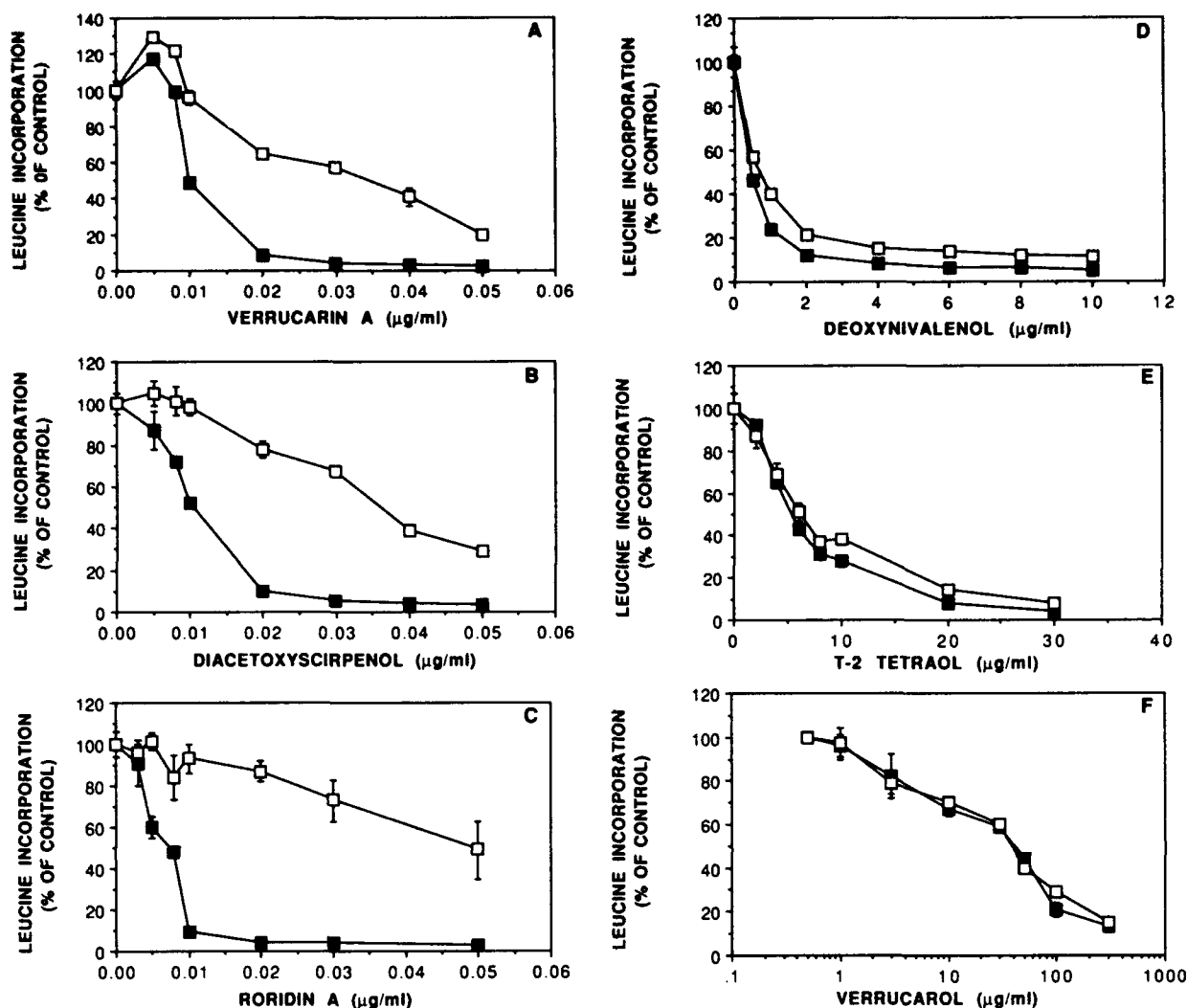


Fig. 5. Effect of emetine preincubation on the response of CHO cells to other trichothecene inhibitors of protein synthesis. A to F, CHO cells were incubated at 37°C with (open symbols) or without (closed symbols) 0.5 µg/ml of emetine. After 60 min, the cells were washed twice with warm HBSS and reincubated in complete H-199 for an additional 1 hr at 37°C. The indicated concentrations of each toxin were then added for 30 min, followed by a 30-min pulse with [<sup>3</sup>H]leucine. The cells were then processed for leucine incorporation as described under "Methods." Data points are the means of triplicate determinations with S.E. The recovery of protein synthesis for each experiment was: A, 41%; B, 48%; C, 60%; D, 43%; E, 48%; F, 41%.

that emetine was an irreversible inhibitor of protein synthesis in HeLa cells. Later, Gupta and Siminovich (1976) demonstrated that emetine was a reversible inhibitor in CHO cells. Still later, Westwood and Wagenaar (1983) reported that emetine was a reversible inhibitor of protein synthesis, but the conditions for its use as such were quite stringent. Within the context of our comparative study, recovery of protein synthesis was examined with concentrations of emetine sufficient to produce maximally sustainable reductions in toxin-cell association at pharmacologically active concentrations of T-2 toxin. Based upon the cumulative data from six experiments (table 1), emetine functioned as a partially reversible inhibitor of protein synthesis from 0.3 to 3.0 µg/ml. However, the most significant recovery of synthesis ( $P < .001$ ) occurred over an even narrower range between 0.3 and 1.0 µg/ml. A concentration of 0.3 µg/ml was the optimum concentration for maximum recovery of synthesis (70%) with near maximum sustainable depression in toxin-cell association (75%). Increasing the emetine concentration beyond 0.5 µg/ml reduced the recovery of synthesis, but did not further improve the depressant effect on

toxin-cell association. The recovery of protein synthesis after incubation with emetine differed significantly ( $P < .05$ ) from the recovery of toxin-cell association at emetine concentrations of 0.3 to 3.0 µg/ml. Again, the most significant differences ( $P < .01$ ) occurred over a very narrow 3-fold range from 0.3 to 1.0 µg/ml (table 1).

The reduction of toxin-cell association by emetine resulted in a modified inhibition response curve for CHO cells subsequently challenged with T-2 toxin (fig. 4A). After emetine preincubation and recovery, the normalized data showed an approximate 4-fold shift in the  $ED_{50}$  for T-2-induced inhibition of protein synthesis. This is consistent with earlier observations (Middlebrook and Leatherman, 1989a), where it was reported that the degree of T-2-induced inhibition of leucine incorporation is proportional to the number of toxin molecules associated with the cells. In contrast to an enzymatically acting toxin such as diphtheria toxin, T-2-induced inhibition of protein synthesis is mediated by a reversible stoichiometric interaction with the ribosomes. For an enzymatically acting toxin, anything short of a complete inhibition of toxin-cell association

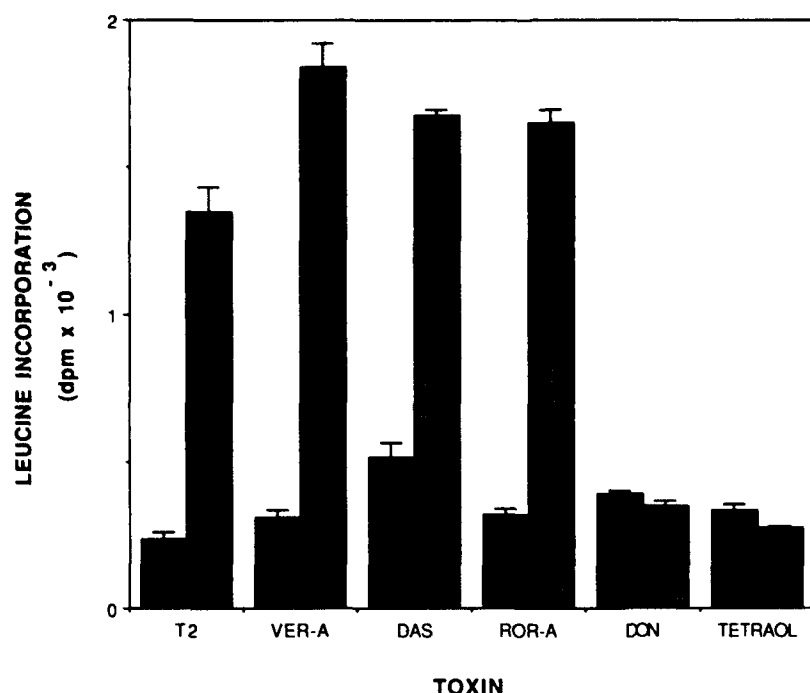


Fig. 6. Leucine incorporation in control and desensitized cells challenged with trichothecene toxins. The data were derived from the experiments described in figures 4A and 5, A to F. These data show actual levels of leucine incorporation in control (solid bars) and desensitized (cross-hatched bars) cells after challenge with the minimum concentration of toxin producing a 95% inhibition of protein synthesis in the control cells. Toxin abbreviations: DAS, diacetoxyscirpenol; VER-A, verrucarrin A; ROR-A, roridin A; DON, deoxynivalenol.

at the cellular level would be of little value for the continued survival of the cell. However, for toxins whose biological activity is mediated by continuously reversible, stoichiometric interactions, reducing the number of intracellular toxin molecules can significantly reduce the biological effects of the toxin. This is particularly true for toxins such as T-2, which have very steep inhibition response curves. As was demonstrated in an earlier investigation, the difference in biological activity between  $0.6 \times 10^6$  and  $2.4 \times 10^6$  toxin molecules per cell was 5 vs. 95% inhibition of protein synthesis (Middlebrook and Leatherman, 1989a).

Cycloheximide, like emetine, stabilizes polyribosomes with an associated decrease in the number of free ribosomes (Oleinick, 1977). Preincubation of the cells with cycloheximide resulted in an inhibition of toxin-cell association that was quantitatively similar to that observed with emetine (fig. 1B). Unlike emetine, cycloheximide's effects on toxin-cell association were completely reversible (fig. 2D), and the inhibition response curves for T-2 toxin in CHO cells were identical in both cycloheximide-treated and nontreated cells (fig. 4B). Within the limits of our investigation, the persistent depression of toxin-cell association and the resulting desensitization of cells to the effects of T-2 toxin were results with specificity for emetine.

This shift in the T-2 inhibition response curve in desensitized cells was not a transient phenomenon secondary to a shift in the kinetic equilibrium between toxin and receptor binding. The desensitizing effects were maintained in emetine-treated cells continuously exposed to T-2 toxin for up to 3 hr (fig. 3). This time was more than sufficient for T-2 toxin-cell association to reach steady-state equilibrium levels (Leatherman and Middlebrook, 1993). In addition, maximum inhibition of toxin-cell association was maintained for up to 8 hr in the absence of extracellular emetine with no indication of diminished efficacy as the toxin concentration was increased (table 2).

The spectrum of emetine's activity was investigated by measuring the response of desensitized and non-desensitized cells to

the inhibitory effects of several other structurally related toxins. Although this was not an exhaustive analysis of the entire class of trichothecenes, one pattern of activity emerged from those toxins which were examined. Emetine modified the response of CHO cells to those trichothecene toxins classified as I-type inhibitors, including T-2 and diacetoxyscirpenol and the macrocyclics, verrucarin A and roridin A (Cundliffe *et al.*, 1974; McLaughlin *et al.*, 1977; Smith *et al.*, 1975). In contrast, we observed no measurable shift in the response of the cells to the inhibitory effects of the less potent (Sato and Ueno, 1977), more polar metabolites deoxynivalenol, verrucarol and T-2 tetraol. Generally, the more polar trichothecene metabolites have been classified as E-type inhibitors because they bind efficiently to polyribosomes (Wei *et al.*, 1974a) and preferentially inhibit translation elongation (Cundliffe *et al.*, 1974; Wei *et al.*, 1974b; McLaughlin *et al.*, 1977). In the accompanying manuscript, evidence is presented indicating that emetine reduced the cellular association of toxin by interfering with the binding of toxin to ribosomes. We proposed that the structural basis for the reduced binding may be the stabilization of ribosomes as polyribosomes with binding configurations nonpermissive for I-type inhibitors. The lack of an emetine effect on trichothecenes which bind preferentially to polysomes (E-type inhibitors) is entirely consistent with that proposal.

In conclusion, emetine can reduce the sensitivity of CHO cells to the toxic effects of T-2 toxin and other I-type inhibitors under conditions that allow a substantial recovery from the toxic effect of emetine on protein synthesis. Further investigation is required to determine whether these results will extrapolate to animal models.

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